Summary Basis for Regulatory Action

Date: November 17, 2011

From: Alain Debrabant, Ph.D., Chairman of the Review Committee

BLA Efficacy Supplement/ STN#: 125361/23

Applicant Name: Abbott Laboratories

Date of Submission: December 22, 2009

FDA Complete Response: October 22, 2010

Date of Re-submission: May 20, 2011

PDUFA Goal Date: November 18, 2011

Trade Name: ABBOTT ESA Chagas

Proper Name: *Trypanosoma cruzi* (*E. coli*, Recombinant) Antigen

Indication: To detect antibodies to *Trypanosoma cruzi* (*T. cruzi*) in human serum and plasma specimens

Recommended Action: Approval

Signatory Authorities Action:

Office's Signatory Authority: Jay S. Epstein, M.D.
Director, OBRR /CBER

□ I concur with the summary review.

□ I concur with the summary review and include a separate review to add further analysis.

□ I do not concur with the summary review and include a separate review.

Material Reviewed/ Consulted: Specific documentation used in developing the SBRA.

Review memos from the following reviewers were used in developing the SBRA.

Discipline reviewed	Reviewer names
Clinical Review	Robert Duncan, Alain Debrabant
Non-Clinical/ Analytical Review	Susan Zullo, Uros Djekic
Statistical Review	Chunrong Cheng
CMC Review	Guang Gao, Laure Juompan
Facility Review	Nicole Trudel, Lori Peters, Qia Bobo, Guang Gao, Alain Debrabant
Biomonitoring Review	Robert Wesley, Janet White, Christine Drabick
Labeling and Promotion	Catherine Miller, Dana Martin
Lot Release Testing	Steve Kerby

Intended Use

The ABBOTT ESA Chagas assay is an in vitro enzyme strip assay intended for the qualitative detection of antibodies to *Trypanosoma cruzi* (*T. cruzi*) in human serum and plasma specimens. The assay is intended for use as an additional, more specific test on human serum or plasma specimens found to be repeatedly reactive using a licensed screening test for antibodies to *T. cruzi*.

Summary and Explanation of the Test

Chagas disease or American Trypanosomiasis is caused by the parasite *T. cruzi*. There are 3 morphologic forms in the life cycle of *T. cruzi*: epimastigote (multiplying form found in the midgut of insect vectors); amastigote (multiplying intracellular form in mammalian hosts); and trypomastigote (non-dividing extracellular form in mammalian blood and insect feces). The majority of *T. cruzi* proteins are expressed in all 3 morphologic forms. The ABBOTT ESA Chagas assay is based on recombinant proteins FP10, FP6, FP3, and TcF. In aggregate, these 4 hybrid recombinant proteins represent at least 14 distinct antigenic regions that broadly represent all 3 morphologic forms. Moreover, these recombinant proteins also contain epitopes recognized by antibodies present in persons with acute *T. cruzi* infections as well as those with chronic Chagas disease.

Biological Principles of the Procedure

The ABBOTT ESA Chagas assay is a multi-step enzyme strip immunoassay.

- Four individually prepared *T. cruzi* recombinant antigens (FP10, FP6, FP3, and TcF), 3 onboard controls (2 onboard visual calibrators [human IgG], and 1 onboard sample addition control [anti-human IgG]) have been applied separately as discrete lines across strips that are composed of nitrocellulose membrane laminated onto a plastic support. These four *T. cruzi* recombinant antigens are also used in the ABBOTT PRISM Chagas assay in which they are coated onto the surface of microparticles. Licensed screening assays use the combined reactivity of their representative antigens to give a single composite signal. In the ABBOTT ESA Chagas, the reactivity of each recombinant antigen is evaluated individually, resulting in a more specific test. The 2 calibrators (H-CAL and L-CAL) are used to interpret results of the assay and indicate that conjugate was added to the strip. The control indicates that a sample was added to the strip.
- The strips are incubated with sample (plasma, serum, or ABBOTT ESA Chagas Positive or Negative Control) and specimen diluent in the trough of the incubation tray. During incubation, *T. cruzi* antibodies present in the sample bind to the antigen(s) on the strips.
- After this first incubation is complete, the strips are washed with a 1x wash buffer. Then a goat anti-human:alkaline phosphatase conjugate is added to the strips and incubated. The conjugate binds antibody to *T. cruzi* that is present.
- After the second incubation is complete, the strips are washed and the enzyme substrate (BCIP/NBT) is added and incubated.

- After incubation with the substrate, the strips are washed and dried. For each sample, color intensity at the location of each recombinant antigen is visually graded by comparing the color intensity of each of the four *T. cruzi* antigens against the color intensity of the L-CAL (visual cutoff, intensity of 1+) and the H-CAL (intensity of 3+) on the strip incubated with the test specimen.

If no antigen bands are visible, or a single antigen band having a +/- intensity (i.e., less than the intensity of the L-CAL) is present, the sample is interpreted as negative (i.e. antibodies to *T. cruzi* are not detected). If two or more bands, with at least one band having an intensity of 1+ or greater are present, the sample is interpreted as positive (i.e. antibodies to *T. cruzi* are detected, indicative of a *T. cruzi* infection). If a single antigen band, having an intensity of 1+ or greater or two or more bands, all having a +/- intensity, are present, the sample is interpreted as indeterminate and must be retested once. If the retest is positive, the sample is interpreted as positive. If the retest is negative or indeterminate, antibodies to *T. cruzi* may or may not be present, and the final interpretation is indeterminate. These individuals, especially those with risk factors for *T. cruzi* infection, may be retested after 6 months using a freshly drawn specimen.

Regulatory Review

Chemistry, Manufacturing and Controls (CMC):

The ABBOTT ESA Chagas assay kit consists of the following components:

- *T. cruzi* (*E. coli*, Recombinant) Antigen Coated Strips. Each strip contains one goat anti-human IgG specimen control band, two human IgG calibrator bands, and four individual bands coated with recombinant *T. cruzi* antigens: FP10, FP6, FP3, and TcF.
- Anti-human (Goat):Alkaline Phosphatase Conjugate
- Specimen Diluent
- Negative Control (recalcified human plasma)
- Positive Control (recalcified human plasma containing antibodies to *T. cruzi*, including a mouse/human chimeric monoclonal antibody to *T. cruzi*)
- Concentrated Wash Buffer
- BCIP/NBT Substrate Tablets
- Incubation Trays

The ABBOTT ESA Chagas assay is manufactured at --- facilities: Abbott Laboratories, Diagnostics Division, Abbott Park, Illinois, ----------
---

----------(b)(4)----------

----------.

1. Preparation of Recombinant Antigens

- (b)(4) *E. coli* expressed recombinant proteins are used in the assay. They include four recombinant proteins expressing *T. cruzi* epitopes, FP3, FP6, FP10, and TcF, used in the *T. cruzi* (*E. coli*, Recombinant) Antigen coated strips ----------(b)(4)----------.
- The test results from three production lots of recombinant antigens were included.
- ----------(b)(4)----------.
- The recombinant antigens were tested and found to be free of contaminating adventitious agents.
- Purity of the antigens was demonstrated by ----------(b)(4)----------. All the antigens met the lot release testing requirements and exhibited lot-to-lot consistency.
- Identity of the recombinant proteins was established by ---(b)(4)---. All the antigens met the lot release testing requirements.
- ----------(b)(4)----------.
- The recombinant antigens passed the validity testing and acceptance criteria.
- These (b)(4) recombinant proteins are also used in the manufacture of the ABBOTT PRISM Chagas assay.

2. Preparation of Anti-Chagas (b)(4) Chimeric Antibody

- A mouse/human chimeric monoclonal anti-Chagas (b)(4) antibody (anti-Chagas (b)(4) chimeric antibody) is used in the manufacture of the kit Positive Control. The (b)(4) epitope is present in the ----(b)(4)---- recombinant antigens.
- ----------(b)(4)----------.
- ----------(b)(4)----------.
- ----------(b)(4)----------.
- ----------(b)(4)----------.
- ----------(b)(4)----------.
- ----------(b)(4)----------.
- ----------(b)(4)----------.
- The chimeric antibody passed the validity testing and acceptance criteria.

- The anti-Chagas (b)(4) chimeric antibody is ----------(b)(4)----------.

3. Preparation of *T. cruzi* (*E. coli*, Recombinant) Antigen Coated Strips

- The four recombinant antigens (FP3, FP6, FP10, and TcF) are -------(b)(4)---------- to prepare four recombinant antigen --(b)(4)-- solutions.
- Goat anti-human IgG and human IgG stocks are ----------(b)(4)---------- in ----(b)(4)---- to prepare three control --(b)(4)-- solutions.
- The seven--(b)(4)-- solutions are (b)(4) onto nitrocellulose to form the four recombinant antigen bands and three control bands on the final strip.
- ----------(b)(4)----------.
- ----------(b)(4)----------.
- ----------(b)(4)----------.
- ----------(b)(4)----------.

4. Preparation of Negative and Positive Controls

- The Negative Control (NC) used in the assay is composed of recalcified non-reactive human plasma.
- The Positive Control (PC) is *T. cruzi* reactive recalcified human plasma diluted in recalcified non-reactive human plasma and mixed with anti-Chagas (b)(4) chimeric antibody.
- ----------(b)(4)---------- testing were performed for Negative and Positive Controls.
- Negative and Positive Controls passed the validity testing and acceptance criteria.

5. Preparation of Other Reagents

- Specimen Diluent and Concentrated Wash Buffer passed both the in process and final testing requirements.
- Conjugate is prepared by diluting a Conjugate Concentrate containing the Anti-human (Goat):Alkaline Phosphatase with Conjugate Diluent containing ----------(b)(4)----------.

6. Final Kit Testing

- ABBOTT ESA Chagas Strips, Conjugate, Concentrated Wash Buffer, NC, PC, BCIP/NBT Substrate Tablets, and Specimen Diluent are ----------(b)(4)----------.
- --(b)(4)—Testing--(b)(4)-- met all the validity and acceptance criteria specifications.
- ----------(b)(4)----------. Final Product Testing meets all the validity and acceptance criteria specifications.

Review Issues

During the review of the CMC section of the BLA Efficacy Supplement the following issues were identified and resolved after further communications with Abbott, review of additional information provided by the firm, and internal discussions:

----------:

----------(b)(4)----------.

----------:

----------(b)(4)----------

----------.

All other minor issues identified during the review of the CMC section of this application have been resolved.

Non-Clinical Studies:

1. Assay Cutoff Justification

The L-CAL band is used as the visual cutoff in the assay. The intensity of the L-CAL band is controlled by a combination of the concentration of human IgG in the ABBOTT ESA Chagas L-CAL -(b)(4)- solution and the concentration of the ABBOTT ESA Chagas Conjugate. For each individual reagent kit master lot, the concentrations of the L-CAL -(b)(4)- solution and the conjugate ----------
----

----------(b)(4)----------

----------. The performance characteristics of the ABBOTT ESA Chagas assay in the non-clinical and clinical studies described below were found acceptable and confirmed the selection of the assay cutoff.

2. Sample Handling and Collection

Abbott performed a series of internal studies to address sample handling and collection. Abbott tested different sample types, ----------(b)(4)----------, specimens collected in serum separator tubes, plasma in various tubes with different anticoagulants (EDTA, potassium oxalate, sodium citrate, ACD-A, ACD-B, CP2D, CPD and CPDA-1), ----------
----------(b)(4)----------
----------.

Abbott tested non-frozen specimens after centrifugation with 30,000-75,000 g-minutes or previously frozen specimens after centrifugation with 30,000- -(b)(4)- g-minutes. Assay results from these studies support the use of the assay with sample types and centrifugation parameters described above. Specimens collected in heparin should not be used due to the possibility of incomplete coagulation in these specimens (stated in the package insert).

3. Potentially Interfering Substances

Spiking Study

No qualitative performance differences were observed when a minimum of 26 nonreactive donor specimens and 27 reactive donor specimens, which were created by spiking with *T. cruzi* antibody to low-level reactivity, were spiked with potentially interfering substances, creating samples with artificially elevated levels of bilirubin (≤ 20 mg/dL), hemoglobin in plasma (≤ 500 mg/dL), red blood cells (≤ 0.4% v/v), triglycerides (≤ 3,000 mg/dL), or protein (≤ 12 g/dL).

This study showed acceptable performance of the ABBOTT ESA Chagas assay with specimens containing up to the concentrations stated above for each of the potentially interfering substances studied.

4. Reproducibility

The reproducibility of the ABBOTT ESA Chagas assay was evaluated in-house at Abbott using three lots by four technicians for three days. The assay was evaluated using a six-member reproducibility panel consisting of three positive samples (Panel Members 1-3), one indeterminate sample (Panel Member 4), and two negative samples (Panel Members 5 and 6). The indeterminate Panel Member 4 was made using *T. cruzi* antibody positive plasma diluted 1:45 with recalcified negative plasma to create a sample with an indeterminate banding pattern (targeting at least 2 bands with intensity of +/-). One replicate of each panel member was tested on the three ABBOTT ESA Chagas Reagent Kit lots for a total of 36 runs (n=36 total replicates per sample). Agreement was 100% for the positive and negative panel members and 75% for the indeterminate panel member, for an overall agreement of 95.8%. In the study, 9 out of 36 strips for the indeterminate Panel Member 4 were interpreted as negative (Table 1). This study shows excellent reproducibility for positive and negative samples and less reliable reproducibility when interpreting indeterminate results.

Table 1: ABBOTT ESA Chagas Assay Reproducibility

Panel Member	Expected Reactivity	Number of Replicates	ABBOTT ESA Chagas			% Agreement
			Positive	Indeterminate	Negative	
1	Positive	36	36	0	0	100.0
2	Positive	36	36	0	0	100.0
3	Positive	36	36	0	0	100.0
4	Indeterminate	36	0	27	9	75.0
5	Negative	36	0	0	36	100.0
6	Negative	36	0	0	36	100.0

5. Specificity

5.1. Study 1: Specificity was determined internally at Abbott. A total of 314 unmatched random donor specimens (157 random serum donor specimens and 157 random plasma donor specimens), presumed negative for antibodies to *T. cruzi*, were tested once using one of three reagent kit lots of ABBOTT ESA Chagas. The final interpretation for five strips with indeterminate results could not be determined (not tested by a *T. cruzi* RIPA). All 309 remaining specimens were negative by ABBOTT ESA Chagas and were not tested by *T. cruzi* RIPA. This study shows acceptable specificity for the ABBOTT ESA Chagas with serum and plasma specimens from blood donors.

5.2. Study 2: Specificity of the ABBOTT ESA Chagas assay was also evaluated internally at Abbott by testing 618 frozen serum and plasma specimens collected from individuals with medical conditions unrelated to *T. cruzi* infection or containing potentially interfering substances (Table 2). Specimens that were positive or indeterminate with the ABBOTT ESA Chagas assay were tested further with a *T. cruzi* RIPA (shown in brackets).

Of the 58 Leishmaniasis specimens tested with ABBOTT ESA Chagas (Table 2), 33 (56.9%) were negative, 24 (41.4%) were indeterminate, and 1 (1.7%) was positive, indicating cross-reactivity of some Leishmaniasis samples with ABBOTT ESA Chagas. The single ABBOTT ESA Chagas positive specimen was negative on *T. cruzi* RIPA. Twenty (20) of the 24 specimens that were indeterminate were not available to be tested using *T. cruzi* RIPA.
NOTE: None of the 25 samples (24 indeterminates and 1 positive) were repeatedly reactive with ABBOTT PRISM Chagas and therefore would not have been tested on ABBOTT ESA Chagas. These specimens were not tested with the *T. cruzi* antibody licensed enzyme-linked immunosorbant assay (*T. cruzi* antibody licensed ELISA). In addition, a study has not been performed to evaluate the ability of the ABBOTT ESA Chagas to resolve specimens from *Leishmania* infected individuals that were repeatedly reactive on the *T. cruzi* antibody licensed ELISA.

Of the 32 Malaria Positive specimens tested with ABBOTT ESA Chagas (Table 2), 29 (90.6%) were negative, and 3 (9.4%) were indeterminate and were also negative on *T. cruzi* RIPA, indicating limited cross-reactivity of some Malaria Positive samples with ABBOTT ESA Chagas. None of the 32 specimens was positive on the ABBOTT ESA Chagas.

Of the remaining 528 specimens tested with ABBOTT ESA Chagas (Table 2), 510 (96.6%) were negative, 18 (3.4%) were indeterminate and none were positive, indicating acceptable specificity of the ABBOTT ESA Chagas with specimens from individuals with medical conditions unrelated to *T. cruzi* infection.

Table 2: Specificity of ABBOTT ESA Chagas with Specimens from Individuals with Medical Conditions Unrelated to *T. cruzi* Infection

Category	ABBOTT ESA Chagas Results		
	Negative	Indeterminate	Positive
Leishmaniasis[a]	33 [0-0-0-33]	24 [2-0-2-20]	1 [0-0-1-0]
Malaria Positive[b]	29 [0-0-0-29]	3 [0-0-3-0]	0 [0-0-0-0]
Syphilis Serologic Positive	15 [0-0-0-15]	1 [0-0-1-0]	0 [0-0-0-0]
Other Medical Conditions Unrelated to *T. cruzi* Infection and Specimens Containing Potentially Interfering Substances[c]	495 [0-0-0-495]	17[d] [3-1-13-0][e]	0 [0-0-0-0]
Total	**572 [0-0-0-572]**	**45 [5-1-19-20]**	**1 [0-0-1-0]**

Note: *T. cruzi* RIPA results are in the bracket: [POS-IND-NEG-ND];

ND = *T. cruzi* RIPA not done.

a These specimens included the following categories: cutaneous (6) and visceral (52).

b These specimens included the following categories: *P falciparum* (16) and *P vivax* (16).

c These 512 specimens included the following categories: anti-HBV positive (16), anti‑HCV positive (16), HBsAg positive (16), anti-HTLV-I/HTLV-II positive (16), anti-HAV positive (16), anti-HIV-1/HIV-2 positive (16), anti-CMV positive (16), anti-EBV positive (16), anti-HSV positive (16), rubella antibody positive (16), West Nile virus antibody positive (16), varicella-zoster virus antibody positive (16), anti-nuclear antibody positive (16), human anti-mouse antibody positive (16), elevated IgG (16), elevated IgM (16), rheumatoid factor positive (16), toxoplasma antibody positive (16), yeast infection (16), tuberculosis positive (16), multiple myeloma (16), monoclonal gammopathy (16), multiparous females (16), elevated triglycerides (16), elevated bilirubin (16), elevated hemoglobin (16), influenza vaccine recipients (50), Dengue virus antibody positive (16), *E. coli* infection (16), and Lyme disease positive (14).

d The 17 ABBOTT ESA Chagas indeterminate specimens from persons with other medical conditions were from the following categories: anti-HTLV-I/HTLV-II positive (2), anti-EBV positive (1), West Nile virus antibody positive (1), varicella-zoster virus antibody positive (2), anti-nuclear antibody positive (1), elevated IgG (1), yeast infection (1), tuberculosis positive (1), elevated triglycerides (1), elevated hemoglobin (1), influenza vaccine recipients (1), Dengue virus antibody positive (2), *E coli* infection (1), and Lyme disease positive (1) .

e The 3 *T. cruzi* RIPA positive specimens from persons with other medical conditions were from the following categories: anti‑HTLV-I/HTLV-II positive (1), *E. coli* infection (1), and influenza vaccine recipients (1). The one *T. cruzi* RIPA indeterminate specimen was from the anti-nuclear antibody positive category.

6. Sensitivity on Geographical Samples

A total of 351 anti-*T. cruzi* positive samples from Argentina, Bolivia, Brazil, Columbia, Chile, Guatemala, Mexico, Nicaragua and the U.S. were evaluated. The specimens were presumed to be positive for anti- *T. cruzi* antibodies by supplier testing and confirmed positive by *T. cruzi* RIPA. Each specimen was tested using ABBOTT PRISM Chagas.

Specimens that were initially reactive by ABBOTT PRISM Chagas were retested in duplicate. Each specimen was tested once across three reagent lots of ABBOTT ESA Chagas. All 351 samples were reactive by ABBOTT PRISM Chagas and confirmed positive for anti- *T. cruzi* antibodies by *T. cruzi* RIPA, and were positive by ABBOTT ESA Chagas.

This study demonstrates acceptable sensitivity of the ABBOTT ESA Chagas with specimens repeatedly reactive on the ABBOTT PRISM Chagas and confirmed positive by *T. cruzi* RIPA.

7. ----------**(b)(4)**----------

----------(b)(4)----------

----------.

----------(b)(4)----------
----------.

---------(b)(4)----------
----------.

----------(b)(4)----------

----------.

----------(b)(4)----------

--(b)(4)--

----------(b)(4)----------

----------.

8. Stability

Real time stability studies were performed on (b)(4) lots of the ABBOTT ESA Chagas Kit for (b)(4) months to determine appropriate storage conditions (2-8^{o}C for the ABBOTT ESA Chagas Assay Kit and 15-30^{o}C for both the 1X Wash Buffer and substrate solution) and expiration dating (shelf life). Abbott showed that the reagents were stable at the recommended storage temperatures for (b)(4) months. Reagents stored inverted with continuous contact with the container closure were stable for up to (b)(4) months at their respective storage temperature. Further, all evaluation criteria were met for reconstituted substrate solution stored at 15-30^{o}C for up to (b)(4) hours and for diluted wash buffer stored at 15-30^{o}C for up to (b)(4) days. These stability studies support the expiration dating of 18 months for the ABBOTT ESA Chagas kit.

9. Microbial Contamination

----------(b)(4)----------

----------.

In summary, a combination of the results from the Antimicrobial Effectiveness study at post-expiration and results of the Microbial Challenge studies demonstrated that through expiration all components with the exception of conjugate were protected from microbial contamination for all organisms. The existing modes of control in the production of the conjugate provide protection from microbial contamination. These studies support the expiration dating of the ABBOTT ESA Chagas Kit to 18 months.

Review Issues

During the review of the non-clinical section of this submission the following significant issue was identified and resolved after further communications with Abbott, review of additional information provided by the firm, and internal discussions:

Issue: Elevated endogenous levels of potentially interfering substances: In addition to the Spiking Study described in section 3 above, Abbott was requested to perform an in-house study to evaluate the performance of the ABBOTT ESA Chagas assay with specimens with elevated endogenous levels of potentially interfering substances (bilirubin, hemoglobin, triglycerides and total protein). In this study, 20 specimens from patients with elevated levels of endogenous hemoglobin in whole blood (16.2 to 18.1 g/dL), 19 specimens with elevated levels of endogenous triglycerides (1,009 to >10,450 mg/dL), 18 specimens with elevated levels of endogenous total protein (9.1 to 11.2 g/dL), and 20 specimens with elevated levels of endogenous bilirubin (5.1 to 11.2 mg/dL) were spiked with *T. cruzi* antibody to target a low level of reactivity. All of these specimens prior to spiking were negative. All of the spiked specimens tested positive. Results showed no interference of these four specimen categories with the ABBOTT ESA Chagas assay. Abbott agreed to include these results in the package insert and this issue was resolved.

All other minor issues identified during the review of the pre-clinical section of this application have been resolved.

Clinical Studies:

1. Clinical Trial Design

The clinical studies were performed in 4 donor screening sites: 3 blood centers and 1 tissue center. In these studies, the performance of the ABBOTT ESA Chagas is compared to a commercially available *T. cruzi* RIPA. Although it is a Laboratory Developed Test, *T. cruzi* RIPA is the current industry standard to determine the most probable antibody status of specimens found to be repeatedly reactive on either of the two licensed blood screening assays for antibodies to *T. cruzi*. However, the performance characteristics of the *T. cruzi* RIPA have not been completely established and this assay is not licensed. Recognizing that the RIPA is not an absolute standard for determination of *T. cruzi* antibody status, some of the discrepant results in the clinical studies will be interpreted in the light of all of the available evidence for those specimens.

2. Clinical Reproducibility

Reproducibility was determined at the clinical testing sites with ABBOTT ESA Chagas by testing a 6-member panel. Panel Members 1 and 2 were *T. cruzi* antibody-negative specimens. Panel Member 3 was a *T. cruzi* antibody-positive specimen diluted 1:45 with recalcified negative plasma to create an indeterminate banding pattern close to the visual cutoff (targeting at least 2 bands with intensity of +/-). Panel Members 4, 5, and 6 were *T. cruzi* antibody-positive specimens. Each panel member was tested once per day over 3 days with each of 3 reagent lots at 4 clinical sites, with 1 technician at each site. In the study, 8 out of 36 strips for the indeterminate Panel Member 3 were interpreted as negative. Table 4 shows the percent agreement for the strip interpretation for each of the panel members.

There were 36 strips tested for each of the 6 panel members (Table 4) with 4 antigen bands per strip for a total of 864 antigen band readings. 99.19% (857/864) of the antigen band readings were within one level of intensity of the band reading reported by the majority of strip readers. 0.81% (7/864) of the antigen band readings for the 3 positive panel members were two levels of intensity higher than the band reading reported by the majority of strip readers.

This study shows acceptable reproducibility performance for the ABBOTT ESA Chagas.

Table 4: ABBOTT ESA Chagas Assay Reproducibility

Panel Member	Expected Reactivity	Number of Replicates	ABBOTT ESA Chagas			% Agreement
			Positive	Indeterminate	Negative	
1	Negative	36	0	0	36	100.00
2	Negative	36	0	0	36	100.00
3	Indeterminate	36	0	28	8	77.78
4	Positive	36	36	0	0	100.00
5	Positive	36	36	0	0	100.00
6	Positive	36	36	0	0	100.00

3. Clinical Specificity on ELISA Non-Reactive Specimens

A total of 330 serum and plasma specimens from United States blood donors were tested with ABBOTT ESA Chagas (Table 5). The specimens were presumed negative for antibodies to *T. cruzi* based on the *T. cruzi* antibody licensed ELISA. Of the 3 specimens with indeterminate ABBOTT ESA Chagas results, 2 were *T. cruzi* RIPA negative and the other specimen was not tested by *T. cruzi* RIPA. There were no false positives with ABBOTT ESA Chagas.

In this study of *T. cruzi* antibody licensed ELISA non-reactive specimens, ABBOTT ESA Chagas was negative for 327 out of 330 specimens (99.1%, with a 95% confidence interval of 97.4% to 99.8%), indeterminate for 3 specimens (0.9%), and positive for none (0.0%).

Table 5: Specificity of ABBOTT ESA Chagas with Specimens from U.S. Blood Donors

	Number	ABBOTT ESA Chagas Results		
Donors	Tested	Positive	Indeterminate	Negative
Plasma	165	0	0	165
Serum	165	0	3	162
Total Donors	330	0	3	327

4. Clinical Studies of U.S. Blood Donor Specimens

4.1. Supplemental Testing of Specimens Repeatedly Reactive by ABBOTT PRISM Chagas

A total of 221 United States blood donor specimens that were repeatedly reactive by the ABBOTT PRISM Chagas were tested with the ABBOTT ESA Chagas assay and with *T. cruzi* RIPA. Of the 221 blood donor specimens, 58 were prospectively identified by testing 41,760 fresh donor specimens and 163 were identified by testing 202 preselected donor specimens that were repeatedly reactive by a *T. cruzi* antibody licensed ELISA.

A comparison of ABBOTT ESA Chagas results with *T. cruzi* RIPA results for the 221 ABBOTT PRISM Chagas repeatedly reactive U.S. blood donor specimens is shown in Table 6. This study shows a high level of concordance of positives (145/146 or 99.3%) on the ABBOTT ESA Chagas with specimens positive by *T. cruzi* RIPA, demonstrating consistency with *T. cruzi* RIPA for samples repeatedly reactive on ABBOTT PRISM Chagas.

One specimen that was ABBOTT ESA Chagas negative and *T. cruzi* RIPA positive (Table 6) was repeatedly reactive by ABBOTT PRISM Chagas and non-reactive by *T. cruzi* antibody licensed ELISA. The follow-up testing results for a new specimen from the same donor were non-reactive by ABBOTT PRISM Chagas (S/CO = 0.85, 0.91, 0.82), non-reactive by *T. cruzi* antibody licensed ELISA (S/CO = 0.094), negative by

ABBOTT ESA Chagas (FP10 -, FP6 +/-, FP3 - and TcF -), and negative by *T. cruzi* RIPA, indicating that this donor was most likely not infected with *T. cruzi* and the negative result on the ABBOTT ESA Chagas for the index donation was correct.

There were 21 specimens that gave a positive result on the ABBOTT ESA Chagas and a negative result on the *T. cruzi* RIPA (Table 6). For further discussion of the interpretation of these specimens see Section 4.3 below

Table 6: Supplemental Testing of U.S. Blood Donor Specimens Repeatedly Reactive by ABBOTT PRISM Chagas

ABBOTT ESA Chagas	*T. cruzi* RIPA			
	Positive	Indeterminate	Negative	Total
Positive	145	5	21	171
Indeterminate	0	0	7	7
Negative	1	0	42	43
Total	146	5	70	221

Of the 41,760 blood donors screened with the ABBOTT PRISM Chagas, 58 were repeatedly reactive (Table 7), and of those, 6 were positive on both the ABBOTT ESA Chagas and *T. cruzi* RIPA, 3 were positive on the ABBOTT ESA Chagas only, and one was positive on *T. cruzi* RIPA only (in Table 6).

Of the 3 ABBOTT ESA Chagas positive and *T. cruzi* RIPA negative specimens, two of the specimens were repeatedly reactive on both screening tests, and had 3 or more reactive bands on the ABBOTT ESA Chagas. One of these specimens came from an individual that was not available for follow-up. The second individual gave a follow-up sample that tested positive by ABBOTT ESA Chagas, indeterminate by *T. cruzi* RIPA, and was high negative (S/CO>0.9) on the two licensed screening assays; however, this donor was born in a Chagas-endemic country. Altogether this evidence suggests that the two donors were true positives. The third specimen was repeatedly reactive on only one screening test, had fewer than 3 reactive bands on the ABBOTT ESA Chagas, and a follow-up specimen was indeterminate on the ABBOTT ESA Chagas, negative on the *T. cruzi* RIPA and non-reactive on the screening tests. Therefore, this 1 out of the 9 ABBOTT ESA Chagas positive results was a false positive.

This study shows concordance (6 out of 6) for ABBOTT ESA Chagas positive results with *T. cruzi* RIPA positive results for prospectively acquired U.S. blood donor specimens found repeatedly reactive by ABBOTT PRISM Chagas.

Table 7: Confirmation of ABBOTT PRISM Chagas Repeatedly Reactive Specimens

Category	Number of Specimens Tested	ABBOTT PRISM Chagas Repeatedly Reactive/Number of Specimens Tested	ABBOTT ESA Chagas Positive/Number of Specimens Tested
U.S. Blood Donors	41,760	58/41,760 (0.14%)	9[a]/58 (15.52%)

[a] Of these 9 ABBOTT ESA Chagas positives, 6 were positive on *T. cruzi* RIPA and 3 were positive on ABBOTT ESA Chagas only. Of these 3, it is likely that 2 were true positives and the third was a false positive.

4.2. Supplemental Testing of Specimens Repeatedly Reactive by a *T. cruzi* Antibody Licensed ELISA

A total of 215 United States blood donor specimens that were repeatedly reactive by the *T. cruzi* antibody licensed ELISA were tested with the ABBOTT ESA Chagas assay and with *T. cruzi* RIPA. Of the 215 blood donor specimens, 13 were from testing 16,292 fresh donor specimens and 202 were from testing preselected donor specimens repeatedly reactive by the *T. cruzi* antibody licensed ELISA.

A comparison of ABBOTT ESA Chagas results with *T. cruzi* RIPA results for the 215 *T. cruzi* antibody licensed ELISA repeatedly reactive U.S. blood donors is shown in Table 8. This study shows a high level of concordance of positives (144/144 or 100.0%) on the ABBOTT ESA Chagas with specimens positive by *T. cruzi* RIPA, demonstrating consistency with *T. cruzi* RIPA for samples repeatedly reactive on the *T. cruzi* antibody licensed ELISA.

There were 22 specimens that gave a positive result on the ABBOTT ESA Chagas and a negative result on the *T. cruzi* RIPA (Table 8). For further discussion of the interpretation of these specimens see Section 4.3 below.

Table 8: Supplemental Testing of U.S. Blood Donor Specimens Repeatedly Reactive by a *T. cruzi* Antibody Licensed ELISA

ABBOTT ESA Chagas	*T. cruzi* RIPA			
	Positive	Indeterminate	Negative	Total
Positive	144	5	22	171
Indeterminate	0	0	3	3
Negative	0	0	41	41
Total	144	5	66	215

Of the 16,292 blood donors screened with the *T. cruzi* antibody licensed ELISA, 13 were repeatedly reactive (Table 9), and of those, 5 were positive on both the ABBOTT ESA Chagas and the *T. cruzi* RIPA, and 2 were positive on the ABBOTT ESA Chagas only.

Of the 2 ABBOTT ESA Chagas positive and *T. cruzi* RIPA negative specimens, both were repeatedly reactive on both screening tests, and had 3 or more reactive bands on the ABBOTT ESA Chagas. One of these specimens came from an individual that was not available for follow-up. The second individual gave a follow-up sample that tested positive by ABBOTT ESA Chagas, indeterminate by *T. cruzi* RIPA, and was high negative (S/CO>0.9) on the two licensed screening assays; however, this donor was born in a Chagas-endemic country. Altogether this evidence suggests that the two donors were true positives.

This study shows concordance (5 out of 5) for ABBOTT ESA Chagas positive results with *T. cruzi* RIPA positive results for prospectively acquired U.S. blood donor specimens found repeatedly reactive by *T. cruzi* antibody licensed ELISA.

Table 9: Confirmation of *T. cruzi* Antibody Licensed ELISA Repeatedly Reactive Specimens

Category	Number of Specimens Tested	*T. cruzi* Antibody Licensed ELISA Repeatedly Reactive/Number of Specimens Tested	ABBOTT ESA Chagas Positive/Number of Specimens Tested
U.S. Blood Donors	16,292	13/16,292 (0.08%)	7[a]/13 (53.85%)

[a] Of these 7 ABBOTT ESA Chagas positives, 5 were positive on *T. cruzi* RIPA and 2 were positive on ABBOTT ESA Chagas only. These 2 were likely true positives.

4.3. Supplemental Testing of Specimens Repeatedly Reactive by the ABBOTT PRISM Chagas and/or the *T. cruzi* Antibody Licensed ELISA

A total of 266 U.S. blood donor specimens were tested with ABBOTT ESA Chagas and *T. cruzi* RIPA. Of these specimens, 202 were preselected donor specimens repeatedly reactive by *T. cruzi* antibody licensed ELISA and 64 specimens were prospectively identified donor specimens that were repeatedly reactive by *T. cruzi* antibody licensed ELISA and/or ABBOTT PRISM Chagas.

A comparison of ABBOTT ESA Chagas results and *T. cruzi* RIPA results for the 266 U.S. blood donors repeatedly reactive on a licensed screening test for *T. cruzi* antibodies from sections 4.1 and 4.2 is shown in Table 10. This study shows a high level of concordance of positives (145/146 or 99.3%) on the ABBOTT ESA Chagas with specimens positive by *T. cruzi* RIPA, demonstrating consistency with *T. cruzi* RIPA for samples repeatedly reactive on ABBOTT PRISM Chagas and/or the *T. cruzi* antibody licensed ELISA.

Of the 266 repeatedly reactive specimens, there were 145 that were positive on both the ABBOTT ESA Chagas and *T. cruzi* RIPA, 5 that were positive on ABBOTT ESA Chagas and indeterminate on *T. cruzi* RIPA, and 23 that were positive on ABBOTT ESA Chagas and negative on *T. cruzi* RIPA (Table 10).

Recognizing that the *T. cruzi* RIPA is not an absolute standard for determination of *T. cruzi* antibody status, some of the discrepant results in these studies were interpreted in the light of all of the available evidence for those specimens. Specimens that were repeatedly reactive on the two licensed screening assays, and showed 3 or more reactive bands on the ABBOTT ESA Chagas were interpreted as true positive. Specimens that were repeatedly reactive on at least one screening test and had a positive ABBOTT ESA Chagas result but with fewer than 3 reactive bands were interpreted as inconclusive. Risk factors for *T. cruzi* infection such as immigration from a Chagas-endemic country and intensity of the bands on the ABBOTT ESA Chagas were also considered in the interpretation of true positives and inconclusives. A negative result on a follow-up sample for both ABBOTT ESA Chagas and *T. cruzi* RIPA were used to reinterpret the true status of the index specimen as negative.

There were 23 specimens that gave positive test results on the ABBOTT ESA Chagas and negative test results on *T. cruzi* RIPA (Table 10). Of these, 19 were repeatedly reactive on both the ABBOTT PRISM Chagas and the *T. cruzi* antibody licensed ELISA and also showed 3 or more bands on the ABBOTT ESA Chagas strips. These 19 specimens can be classified as most likely true positives on the ABBOTT ESA Chagas. For 3 other specimens out of these 23, none met all criteria for true positivity; however, they showed sufficient reactivity to be interpreted as inconclusive with regard to their *T. cruzi* antibody status. The remaining 1 out of these 23 was non-reactive on one of the licensed screening assays, and can be classified as false positive on the ABBOTT ESA Chagas based on a follow-up sample that tested non-reactive on both licensed screening assays and negative on the ABBOTT ESA Chagas and *T. cruzi* RIPA.

There were 5 specimens that gave a positive result on the ABBOTT ESA Chagas and an indeterminate result on the *T. cruzi* RIPA (Table 10). Considering that all 5 of these were repeatedly reactive on both the ABBOTT PRISM Chagas and the *T. cruzi* antibody licensed ELISA and 4 of the 5 showed 3 or more bands on the ABBOTT ESA Chagas strips and 3 of the donors came from Chagas-endemic countries, at least 4 of these 5 specimens can be classified as most likely true positives on the ABBOTT ESA Chagas. The fifth specimen, which was also repeatedly reactive on both licensed screening tests but showed only 2 bands on the ABBOTT ESA Chagas strip can be classified as inconclusive with respect to its *T. cruzi* antibody status.

There were 10 specimens that gave an indeterminate result on the ABBOTT ESA Chagas and a negative result on the *T. cruzi* RIPA (Table 10). Considering that all of these were repeatedly reactive on only one of the two licensed screening tests, 3 of them had 3 or more low-intensity reactive bands and 7 had fewer than 3 reactive bands on the ABBOTT ESA Chagas strips, the most likely interpretation of the *T. cruzi* antibody status for these 10 specimens is that 3 were inconclusive and 7 were true negatives.

In conclusion, based on this analysis, out of 168 true positives there were 168 (100%, with a 95% confidence interval of 97.8% to 100.0%) that were positive on the ABBOTT ESA Chagas. These data demonstrate high sensitivity of ABBOTT ESA Chagas on repeatedly reactive samples, with sensitivity greater than *T. cruzi* RIPA.

In addition, ABBOTT ESA Chagas was negative for 83 out of 91 specimens with a status interpreted as true negative (91.2%, with a 95% confidence interval of 83.4% to 96.1%). These data demonstrate specificity comparable to *T cruzi* RIPA.

Table 10: Supplemental Testing of Specimens Repeatedly Reactive by a Licensed Screening Test for Antibodies to *T. cruzi* from Table 8 and Table 9

ABBOTT ESA Chagas	*T. cruzi* RIPA			
	Positive	Indeterminate	Negative	Total
Positive	145	5[b]	23[c]	173
Indeterminate	0	0	10[d]	10
Negative	1[a]	0	82	83
Total	146	5	115	266

[a] One specimen that was ABBOTT ESA Chagas negative and *T. cruzi* RIPA positive was a false positive on *T. cruzi* RIPA.
[b] Additional analysis of test results and other information for these 5 specimens has led to the conclusion that 4 were true positives on ABBOTT ESA Chagas and 1 was inconclusive.
[c] Additional analysis of test results and other information for these 23 specimens has led to the conclusion that 19 were true positives on ABBOTT ESA Chagas, 3 were inconclusive, and 1 was a false positive on ABBOTT ESA Chagas.
[d] Additional analysis of test results and other information for these 10 specimens has led to the conclusion that 7 of them were false indeterminates on ABBOTT ESA Chagas, and were true negatives. The other 3 remained inconclusive concerning their final interpretation.

5. Clinical Sensitivity in Non-U.S. Specimens

5.1. Parasitologically Confirmed Specimens

A total of 110 serum specimens from individuals known to be positive for the *T. cruzi* parasite were tested with the ABBOTT PRISM Chagas and the ABBOTT ESA Chagas assay (Table 11). Of the 110 specimens, 65 were from individuals that tested positive by identification of the parasite with xenodiagnosis. The remaining 45 specimens were from individuals known to be positive for the *T. cruzi* parasite by historical identification of the parasite with xenodiagnosis or hemoculture. The specimens were obtained from the Chagas-endemic countries of Argentina, Bolivia, Brazil, and Peru. All 110 specimens were repeatedly reactive on the ABBOTT PRISM Chagas and were positive for *T. cruzi* antibodies with the ABBOTT ESA Chagas assay (Table 11).

Table 11: Sensitivity of ABBOTT ESA Chagas with Specimens Parasite Positive for *T. cruzi*.

Category	Number Tested	ABBOTT ESA Chagas Results		
		Positive	Indeterminate	Negative
Preselected *T. cruzi* Parasite Positive	110	110	0	0

5.2. Serologically Confirmed Specimens

A total of 85 serum specimens from individuals reactive for *T. cruzi* antibodies based on 2 different serologic tests for antibodies to *T. cruzi* (i.e., ELISA, immunofluorescence assay [IFA], or indirect hemagglutination assay [IHA]), were obtained from Argentina and were tested with the ABBOTT PRISM Chagas and with the ABBOTT ESA Chagas assay. All 85 specimens were repeatedly reactive on the ABBOTT PRISM Chagas and were positive for *T. cruzi* antibodies with a *T. cruzi* RIPA and with the ABBOTT ESA Chagas assay (Table 12).

Table 12: Sensitivity of ABBOTT ESA Chagas with Preselected *T. cruzi* Serologic Positive Specimens

Category	Number Tested	ABBOTT ESA Chagas Results		
		Positive	Indeterminate	Negative
Preselected *T. cruzi* Serologic Positive, South America	85	85	0	0

These studies in sections 5.1 and 5.2 showed that for previously confirmed non-U.S. specimens from Chagas-endemic areas, the ABBOTT ESA Chagas demonstrated 100% sensitivity.

6. Prospective Studies in High Risk Populations

A total of 524 serum specimens from individuals residing in Chagas-endemic areas were tested with ABBOTT ESA Chagas, ABBOTT PRISM Chagas, and a *T. cruzi* antibody licensed ELISA. Specimens were obtained from Argentina, Brazil, Guatemala, Panama, and Peru. Specimens that were positive and specimens that were indeterminate with ABBOTT ESA Chagas and/or repeatedly reactive with either ABBOTT PRISM Chagas or a *T. cruzi* antibody licensed ELISA, if available, were tested further with *T. cruzi* RIPA.

ABBOTT ESA Chagas results and *T. cruzi* RIPA results (shown in brackets) for the 524 High Risk specimens screened using the ABBOTT PRISM Chagas are shown in Table 13. One (1) specimen that was non-reactive using ABBOTT PRISM Chagas was positive by both the ABBOTT ESA Chagas and *T. cruzi* RIPA.

Table 13: High Risk Chagas Endemic Specimens Tested with ABBOTT PRISM Chagas and ABBOTT ESA Chagas

ABBOTT PRISM Chagas	ABBOTT ESA Chagas			Total
	Positive	Indeterminate	Negative	
Repeatedly Reactive	137 [129-3-5][a]	0	0	137
Non-reactive	10 [1-0-9][a]	21[b] [2-0-12][a]	356[c] [0-0-2][a]	387
Total	147	21	356	524

[a] *T. cruzi* RIPA: [POS-IND-NEG]

[b] Out of 21 specimens that tested indeterminate with the ABBOTT ESA Chagas assay, 7 specimens were not available for testing by *T. cruzi* RIPA.

[c] *T. cruzi* RIPA testing was not performed for 354 out of 356 non-reactive specimens.

ABBOTT ESA Chagas results and *T. cruzi* RIPA results (shown in brackets) for the 524 High Risk specimens screened using the *T. cruzi* antibody licensed ELISA is shown in Table 14. Two (2) specimens that were non-reactive using the *T. cruzi* antibody licensed ELISA were positive by both the ABBOTT ESA Chagas and *T. cruzi* RIPA.

Table 14: High Risk Chagas Endemic Specimens Tested with a *T. cruzi* Antibody Licensed ELISA and ABBOTT ESA Chagas

T. cruzi Antibody Licensed ELISA	ABBOTT ESA Chagas			Total
	Positive	Indeterminate	Negative	
Repeatedly Reactive	139 [128-3-8][a]	1 [1-0-0][a]	2 [0-0-2][a]	142
Non-reactive	8 [2-0-6][a]	20[b] [1-0-12][a]	354[c]	382
Total	147	21	356	524

[a] *T. cruzi* RIPA: [POS-IND-NEG]

[b] Out of 20 specimens that tested indeterminate with the ABBOTT ESA Chagas assay, 7 specimens were not available for testing by *T. cruzi* RIPA

[c] *T. cruzi* RIPA testing was not performed for 354 non-reactive specimens.

A comparison of ABBOTT ESA Chagas results and *T. cruzi* RIPA results for the 524 High Risk specimens is shown in Table 15. This study shows a high level of concordance of positives (130/132 or 98.5%) on the ABBOTT ESA Chagas with specimens positive by *T. cruzi* RIPA, demonstrating consistency with *T. cruzi* RIPA for samples repeatedly reactive on ABBOTT PRISM Chagas and/or the *T. cruzi* antibody licensed ELISA.

Recognizing that the *T. cruzi* RIPA is not an absolute standard for determination of *T. cruzi* antibody status, discrepant results on ABBOTT ESA Chagas and *T. cruzi* RIPA were reinterpreted according to the criteria outlined in Section 4.3.

There were 14 specimens that gave a positive result on the ABBOTT ESA Chagas and a negative result on *T. cruzi* RIPA (Table 15). Considering that these specimens were

collected in Chagas-endemic countries, and that 4 out of these 14 were repeatedly reactive on both the ABBOTT PRISM Chagas and the *T. cruzi* antibody licensed ELISA and also showed 3 or more bands on the ABBOTT ESA Chagas strips, these 4 specimens can be classified as most likely true positives on the ABBOTT ESA Chagas. Four (4) other specimens out of these 14 were repeatedly reactive on one of the 2 licensed screening assays and also showed 3 or more bands on the ABBOTT ESA Chagas strip. Therefore, these 4 specimens can be classified as inconclusive with regard to their *T. cruzi* antibody status. The remaining 6 out of these 14 were non-reactive on both of the licensed screening assays, and can be classified as false positives on the ABBOTT ESA Chagas.

There were 3 specimens that gave a positive result on the ABBOTT ESA Chagas and an indeterminate result on the *T. cruzi* RIPA (Table 15). Considering that these specimens came from Chagas-endemic countries, and that all 3 of these were repeatedly reactive on both the ABBOTT PRISM Chagas and the *T. cruzi* licensed antibody ELISA and also showed 3 or more bands on the ABBOTT ESA Chagas strips, these 3 specimens can be classified as most likely true positives on the ABBOTT ESA Chagas.

There were 21 specimens that gave an indeterminate result on the ABBOTT ESA Chagas, and of those, 2 gave a positive result, 12 gave a negative result, and 7 were not tested on *T. cruzi* RIPA (Table 15). Considering that one of the 2 specimens that was *T. cruzi* RIPA positive was repeatedly reactive on only one screening test, and had only 2 low-intensity reactive bands on the ABBOTT ESA Chagas, its antibody status is inconclusive at best. The other specimen that gave a positive result on the *T. cruzi* RIPA was non-reactive on both screening assays and had only 2 low-intensity reactive bands on the ABBOTT ESA Chagas; therefore, its most likely antibody status is negative. Considering that all of the 12 that gave a negative result on the *T. cruzi* RIPA and the 7 that were not tested on the *T. cruzi* RIPA were non-reactive on both screening assays, and only one of them had 3 low-intensity reactive bands on the ABBOTT ESA Chagas, the most likely interpretation of the *T. cruzi* antibody status of these 19 specimens is negative.

In conclusion, based on this analysis, out of 137 true positives there were 137 (100%, with a 95% confidence interval of 97.3% to 100.0%) that were positive on ABBOTT ESA Chagas. These data demonstrate high sensitivity of ABBOTT ESA Chagas on repeatedly reactive samples, with sensitivity comparable to *T. cruzi* RIPA.

In addition, ABBOTT ESA Chagas was negative for 356 out of 382 specimens with a status interpreted as true negative (93.2%, with a 95% confidence interval of 90.2% to 95.5%). These data demonstrate specificity comparable to *T cruzi* RIPA.

Table 15: High Risk Chagas Endemic Specimens Tested with ABBOTT ESA Chagas and a *T. cruzi* RIPA

ABBOTT ESA Chagas	*T. cruzi* RIPA				
	Positive	Indeterminate	Negative	Not Tested	Total
Positive	130	3[b]	14[c]	0	147
Indeterminate	2[a]	0	12[d]	7[d]	21
Negative	0	0	2	354	356[e]
Total	132	3	28	361	524

[a] Additional analysis of test results for these 2 specimens has led to the conclusion that one of these was inconclusive and the other was negative.

[b] Additional analysis of test results for these 3 specimens has led to the conclusion that all of these 3 were true positives on ABBOTT ESA Chagas.

[c] Additional analysis of test results for these 14 specimens has led to the conclusion that 4 were true positives on ABBOTT ESA Chagas, 4 were inconclusive, and 6 were false positives on ABBOTT ESA Chagas.

[d] Additional analysis of test results for these 19 specimens has led to the conclusion that all 19 of these were false indeterminates on the ABBOTT ESA Chagas, and were true negatives.

[e] Of the 356 specimens negative by ABBOTT ESA Chagas, 354 specimens were non-reactive by both ABBOTT PRISM Chagas and *T. cruzi* antibody licensed ELISA and not tested by *T. cruzi* RIPA. Two specimens were non-reactive by ABBOTT PRISM Chagas and repeatedly reactive by *T. cruzi* antibody licensed ELISA and were *T. cruzi* RIPA negative.

7. Bioresearch Monitoring Inspection (BIMO Inspection)

----------(Removed Per Privacy Act)----------:

(Removed Per Privacy Act)

----------(Removed Per Privacy Act)----------.

Review Issues

During the review of the clinical section of the BLA Efficacy Supplement the following significant issues were identified and resolved after further communications with Abbott, review of additional information provided by the firm, and internal discussions:

Issue 1: Interpretation of single band results:
In the original interpretation of the results, a specimen showing no band or a single band of any intensity on the ABBOTT ESA Chagas assay was interpreted as negative. FDA was concerned about interpreting a single band result with band intensity of 1+ and above (i.e., equal to or greater than the L-CAL, used as a cutoff in the assay) as a negative result for the following reasons: 1) each of the four recombinant proteins painted on the strips contains several different *T. cruzi* specific epitopes. Any single band result with intensity equal or greater than the visual cutoff (1+) could indicate significant reactivity to one or more *T. cruzi* specific epitopes, and 2) a significant number of *Leishmania* and malaria specimens tested on this assay gave single band results. With this level of cross-reactivity, it is possible that a number of single band results that have band intensity equal to or greater than 1+ might indicate *Leishmania* or malaria infection. Therefore, FDA recommended that strips with only one band of 1+ intensity or greater should be interpreted as indeterminate. Only results with no band or a single band with a band intensity of +/- should be interpreted as negative.

Abbott re-analyzed the results of the pre-clinical and clinical studies using the revised interpretation for single band results. An additional 48 specimens in the non-clinical studies and 26 specimens from the clinical studies that were interpreted as negative by the ABBOTT ESA Chagas assay became indeterminate as a result of the revised interpretation of the single band results. A consequence of the re-analysis of the clinical trial data is that *T. cruzi* RIPA results were not available for the some of newly indeterminate specimens because they did not qualify for *T. cruzi* RIPA testing based on the testing algorithm used in the clinical trial and the specimens were destroyed.

Review of the data showed an acceptable increase in the number of indeterminate results and an acceptable performance of the assay as illustrated in the non-clinical and clinical sections above. This issue was resolved.

Issue 2: Interpretation of indeterminate results:
During the clinical trial, a specimen with an indeterminate result on the ABBOTT ESA Chagas assay was re-tested in duplicate. If both of the duplicate retests were positive, the specimen was interpreted as positive, if one retest was positive and one retest was negative or at least one retest was indeterminate, the specimen was interpreted as indeterminate, and if both of the duplicate retests were negative, the specimen was interpreted as negative. Out of a total of 1,377 specimens tested by ABBOTT ESA Chagas in the clinical studies, 24 (1.74%) were initially indeterminate, and of those, 13 remained indeterminate, 9 became negative, and 2 became positive following retesting in duplicate. The two (2) initially indeterminate specimens that became positive following retesting had each of the two retest strips interpreted as positive by two different readers. Based on these results, FDA recommended that an indeterminate result be retested only once. If the retest is positive, the specimen should be interpreted as positive and if the retest is negative or indeterminate, the specimen should be interpreted as indeterminate, and the package insert should state that these individuals, especially those with risk factors for *T. cruzi* infection, may be retested after 6 months using a freshly drawn specimen. Retesting the same specimen one time will provide additional assurance on

the indeterminate status of this specimen, and retesting the individual after 6 months using a freshly drawn specimen is consistent with other supplemental test package inserts (e.g. HIV western blots, HCV RIBA).

Abbott re-analyzed the results of their pre-clinical and clinical studies using the revised interpretation for indeterminate results. In the non-clinical studies, 7 specimens became indeterminate as a result of single retest revised interpretation. Of the 1,377 specimens tested in the clinical studies, 9 specimens became indeterminate due to the single retest revised interpretation, resulting in a total of 50 indeterminate specimens out of 1,377 total specimens (3.63%). These results showed an acceptable rate of indeterminate results (3.63%) when testing clinical specimens with the ABBOTT ESA Chagas. The package insert was modified to reflect this revised interpretation of the indeterminate results and this issue was resolved.

Issue 3: Postmarket commitment:

----------:

----------(b)(4)----------

----------.

----------(b)(4)----------

----------.

----------:

----------(b)(4)----------

----------(b)(4)----------

----------.

All other minor issues identified during the review of the clinical section of the BLA Efficacy Supplement have been resolved.

Establishment Description:

The ABBOTT ESA Chagas assay is manufactured at (b)(4) facilities: Abbott Laboratories, Diagnostics Division ----------
---
----------(b)(4)----------

----------.

1. Establishment Inspection

1.1. Abbott Park, Illinois

The ABBOTT PRISM Chagas screening assay was approved to be used in the ABBOTT PRISM instrument in April, 2010 under BLA STN 125361/0. The purified recombinant antigens, ----------(b)(4)----------used in the manufacture of the ABBOTT ESA Chagas assay are the same as approved in BLA STN 125361/0. In addition, based on the previous inspection reports supporting the overall compliance status of the license holder, the pre-approval inspection for the Abbott facilities associated with this BLA Efficacy Supplement was waived.

----------(b)(4)----------

----------.

----------(b)(4)----------

---.

----------(b)(4)----------
---.

2. Environmental Impact Analysis, Claims for Categorical Exclusion

Abbott Laboratories has requested that the requirement for an Environmental Assessment be waived in the subject BLA Efficacy Supplement. Under 21 CFR 25.31(c), the requirement for an environmental impact assessment is categorically waived for an application to market a biologic product if the substances associated with that product occur naturally in the environment and the action of the product does not significantly alter the concentration or distribution of those substances, their metabolites, or degradation products in the environment. Abbott Laboratories confirmed that these conditions apply to their subject biologic. The biologic product is antibody-based, is composed of naturally occurring substances, and appears to meet the applicable exclusion criteria under 21 CFR 25.31(c); there is no information indicating that extraordinary circumstances exist. This claim for categorical exclusion was found to be justified.

Lot Release Testing:

In preparation for implementing a Lot Release Testing Plan at approval, a review was performed of the submission and subsequent amendments for supporting information contributing to the establishment of the lot release criteria. All of the studies submitted for specific performance characteristics of the assay (sensitivity, specificity, reproducibility, etc.) were found to be acceptable. Abbott also presented a draft Lot Release Protocol which was found acceptable after minor changes.

Three lots of ABBOTT ESA Chagas kits were received and tested in the Division of Biological Standards and Quality Control (DBSQC), formerly the Division of Product Quality (DPQ). The samples were evaluated using Test Method 000232. All three lots met the current Expected Reactivity for the FDA/CBER Reference Panel used to evaluate kit function. The following is a list of the lots submitted in support of approval:

Master Lot Number	Expiration Date	Lot Release Assay Determination
A00000102	05/25/2011	PASS
A00000202	05/25/2011	PASS
A00000300	04/28/2012	PASS

The three kit lots were manufactured at similar scale and under the same conditions. These lots were tested by Abbott with their internal lot release panel and with the CBER Chagas Lot Release Panel. These testing results demonstrate lot-to-lot consistency for the manufacture of the ABBOTT ESA Chagas assay.

The CBER Chagas Lot Release Panel is currently in use for lot release testing of the two licensed screening tests. This panel consists of 10 members: 4 have an expected reactivity of "+"; 4 have an expected reactivity of "-/+"; and 2 have an expected reactivity of "-". This panel is considered to be suitable as a lot release panel for the ABBOTT ESA Chagas.

Recommendation:

Based on the review of the information submitted in the original application and additional information submitted in response to information requests, all review issues raised by all review committee members have been resolved. I recommend approval of the ABBOTT ESA Chagas to be contingent on Postmarketing Commitments ----------
---

----------(b)(4)----------

----------.